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PCT/US2004/034501
IAP20 Rec'd PCT/PTO 21 APR 2006

ALKYNES III

Field of the invention

The present invention is directed to novel compounds, to a process for their preparation, their use in therapy and pharmaceutical compositions comprising the novel compounds.

Background of the invention

The metabotropic glutamate receptors (mGluR) are G-protein coupled receptors that are involved in the regulation and activity of many synapses in the central nervous system (CNS). Eight metabotropic glutamate receptor subtypes have been identified and are subdivided into three groups based on sequence similarity. Group I consists of mGluR1 and mGluR5. These receptors activate phospholipase C and increase neuronal excitability. Group II, consisting of mGluR2 and mGluR3 as well as group III, consisting of mGluR4, mGluR6, mGluR7 and mGluR8 are capable of inhibiting adenylyl cyclase activity and reduce synaptic transmission. Several of the receptors also exist in various isoforms, occurring by alternative splicing (*Chen, C-Y et al., Journal of Physiology (2002), 538.3, pp. 773-786; Pin, J-P et al., European Journal of Pharmacology (1999), 375, pp. 277-294; Bräuner-Osborne, H et al. Journal of Medicinal Chemistry (2000), 43, pp. 2609-2645; Schoepp, D.D, Jane D.E. Monn J.A. Neuropharmacology (1999), 38, pp. 1431-1476*).

The lower esophageal sphincter (LES) is prone to relaxing intermittently. As a consequence, fluid from the stomach can pass into the esophagus since the mechanical barrier is temporarily lost at such times, an event hereinafter referred to as "reflux".

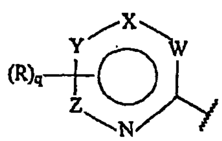
Gastro-esophageal reflux disease (GERD) is the most prevalent upper gastrointestinal tract disease. Current pharmacotherapy aims at reducing gastric acid secretion, or at neutralizing acid in the esophagus. The major mechanism behind reflux has been considered to depend on a hypotonic lower esophageal sphincter. However, e.g. *Holloway & Dent (1990)*

Gastroenterol. Clin. N. Amer. 19, pp. 517-535, has shown that most reflux episodes occur during transient lower esophageal sphincter relaxations (TLESRs), i.e. relaxations not triggered by swallows. It has also been shown that gastric acid secretion usually is normal in patients with GERD.

The problem underlying the present invention was to find new compounds useful in the treatment of GERD.

WO 01/16121 A1 discloses a compound A-L-B, where

A is a 5-, 6- or 7-membered heterocycle

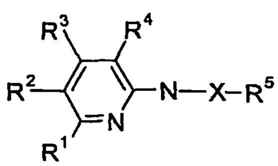


;

L is an alkenylene, alkynylene or azo; and

B is a hydrocarbyl; cyclohydrocarbyl; heterocycle (optionally containing one or more double bonds); or aryl. These compounds have been described as being useful in inter alia cerebral ischemia, chronic neurodegeneration, psychiatric disorders, epilepsy and diseases of the pulmonary system as well as the cardiovascular system.

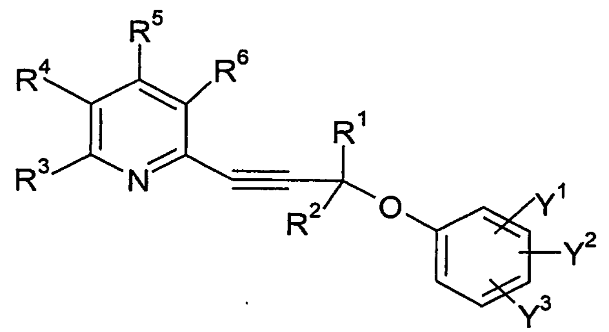
WO 99/02497 A2 discloses compounds of the formula



wherein X may be an alkenylene or an alkynylene bonded via vicinal unsaturated carbon atoms, or an azo group; and R^5 may be an aromatic or heteroaromatic group. These compounds have been described as being useful in inter alia epilepsy, cerebral ischemia and Alzheimer's disease.

Outline of the invention

The present invention is directed to novel compounds according to the general formula I:



(I)

wherein

R^1 is selected from hydrogen, C_1-C_4 alkyl, C_3-C_6 cycloalkyl, aryl and heteroaryl, wherein the aryl or heteroaryl may be substituted by C_1-C_4 alkyl;

R^2 is selected from hydrogen and C_1-C_4 alkyl;

R^3 is selected from hydrogen, C_1-C_4 alkyl, F, CF_3, CHF_2 and CH_2F;

R^4 is selected from hydrogen, F, CF_3, CHF_2, CH_2F and CH_3;

R^5 is selected from hydrogen and F;

R^6 is selected from hydrogen and F;

Y^1 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; benzyloxy; nitro in the meta or para position; and C_1-C_4 alkyl ester;

Y^2 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; and C_1-C_4 alkyl ester;

Y^3 is selected from hydrogen; halogen; nitrile; C_1-C_4 alkoxy; C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom; and C_1-C_4 alkyl ester; or

Y^1 and Y^2 may form an aromatic or non-aromatic ring, optionally substituted by halogen, nitrile, C_1-C_4 alkoxy, C_1-C_4 alkyl wherein one or more of the hydrogen atoms of the alkyl group may be substituted for a fluorine atom, benzyloxy or C_1-C_4 alkyl ester;
with the proviso that when Y^1 is hydrogen, Y^2 is selected from halogen, nitrile, C_1-C_4 alkoxy, and C_1-C_4 alkyl;
as well as pharmaceutically acceptable salts, hydrates, isoforms and/or optical isomers thereof.

The general terms used in the definition of formula I have the following meanings:

Halogen is chloro, fluoro, bromo or iodo.

C_1-C_4 alkyl is a straight or branched alkyl group, each independently containing 1, 2, 3 or 4 carbon atoms, for example methyl, ethyl, n-propyl, n-butyl or isopropyl. In one embodiment, the alkyl groups may contain one or more heteroatoms selected from O, N and S. Examples of such groups are methyl-ethylether, methyl-ethylamine and methyl-thiomethyl.

Cycloalkyl is a cyclic alkyl, each independently containing 3, 4, 5 or 6 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl or cyclohexyl.

C_1-C_4 alkoxy is an alkoxy group containing 1, 2, 3 or 4 carbon atoms, such as methoxy, ethoxy, n-propoxy, n-butoxy or isopropoxy.

The herein used term aryl means aromatic rings with 6-14 carbon atoms including both single rings and polycyclic compounds, such as phenyl, benzyl or naphtyl.

The term heteroaryl as used herein means aromatic rings with 5-14 carbon atoms, including both single rings and polycyclic compounds, such as imidazopyridine, in which

one or several of the ring atoms is either oxygen, nitrogen or sulphur, such as furanyl or thiophenyl.

Within the scope of the invention are also pharmaceutically acceptable salts of the compounds of formula I as well as isomers, hydrates and isoforms thereof.

Pharmaceutically acceptable salts of the compound of formula I are also within the scope of the present invention. Such salts are for example salts formed with mineral acids such as hydrochloric acid; alkali metal salts such as sodium or potassium salts; or alkaline earth metal salts such as calcium or magnesium salts.

The novel compounds according to the present invention are useful in therapy. In one aspect of the invention said compounds are useful for the inhibition of transient lower esophageal sphincter relaxations (TLESRs) and thus for treatment or prevention of gastro-esophageal reflux disorder (GERD). In further embodiments, the compounds according to the present invention are useful for the prevention of reflux, treatment or prevention of regurgitation, treatment or prevention of asthma, treatment or prevention of laryngitis, treatment or prevention of lung disease and for the management of failure to thrive.

A further aspect of the invention is the use of a compound according to formula I, for the manufacture of a medicament for the inhibition of transient lower esophageal sphincter relaxations, for the treatment or prevention of GERD, for the prevention of reflux, for the treatment or prevention of regurgitation, treatment or prevention of asthma, treatment or prevention of laryngitis, treatment or prevention of lung disease and for the management of failure to thrive.

A further aspect of the invention is the use of a compound according to formula I for the manufacture of a medicament for the treatment or prevention of functional gastrointestinal disorders, such as functional dyspepsia (FD). Yet another aspect of the invention is the use of a compound according to formula I for the manufacture of a medicament for the

treatment or prevention of irritable bowel syndrome (IBS), such as constipation predominant IBS, diarrhea predominant IBS or alternating bowel movement predominant IBS.

Still a further aspect of the invention is a method for the treatment of any one of the conditions mentioned above, whereby a pharmaceutically effective amount of a compound according to formula I above, is administered to a subject suffering from said condition(s).

In one aspect of the invention, the compounds of formula I are useful for the treatment and/or prevention of acute and chronic neurological and psychiatric disorders, anxiety and chronic and acute pain disorders. In a further aspect, said compounds are useful for the prevention and/or treatment of pain related to migraine, inflammatory pain, neuropathic pain disorders such as diabetic neuropathies, arthritis and rheumatoid diseases, low back pain, post-operative pain and pain associated with various conditions including cancer, angina, renal or billiary colic, menstruation, migraine and gout.

The term “isomers” is herein defined as compounds of formula I, which differ by the position of their functional groups and/or orientation. By “orientation” is meant stereoisomers, diastereoisomers, regioisomers and enantiomers.

The term “isoforms” as used herein is defined as compounds of formula I which differ by their crystal lattice, such as crystalline compounds and amorphous compounds.

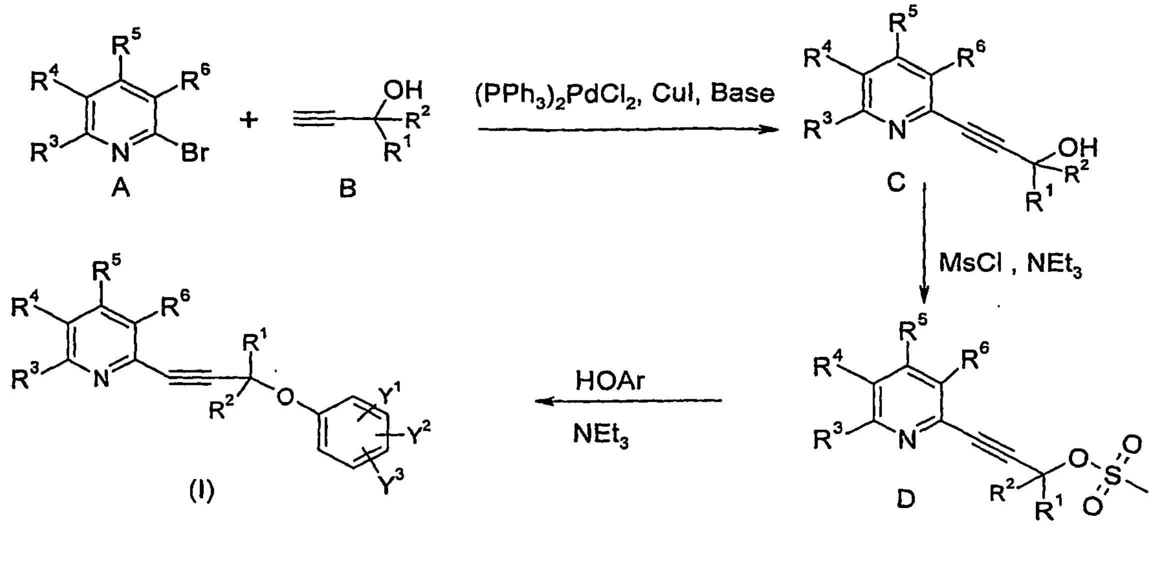
The wording “TLESR”, transient lower esophageal sphincter relaxations, is herein defined in accordance with *Mittal, R.K., Holloway, R.H., Penagini, R., Blackshaw, L.A., Dent, J., 1995; Transient lower esophageal sphincter relaxation. Gastroenterology 109, pp. 601-610.*

The wording “reflux” is defined herein as fluid from the stomach being able to pass into the esophagus, since the mechanical barrier is temporarily lost at such times.

The wording "GERD", gastro-esophageal reflux disease, is defined herein in accordance with *van Heerwarden, M.A., Smout A.J.P.M., 2000; Diagnosis of reflux disease. Baillière's Clin. Gastroenterol. 14, pp. 759-774.*

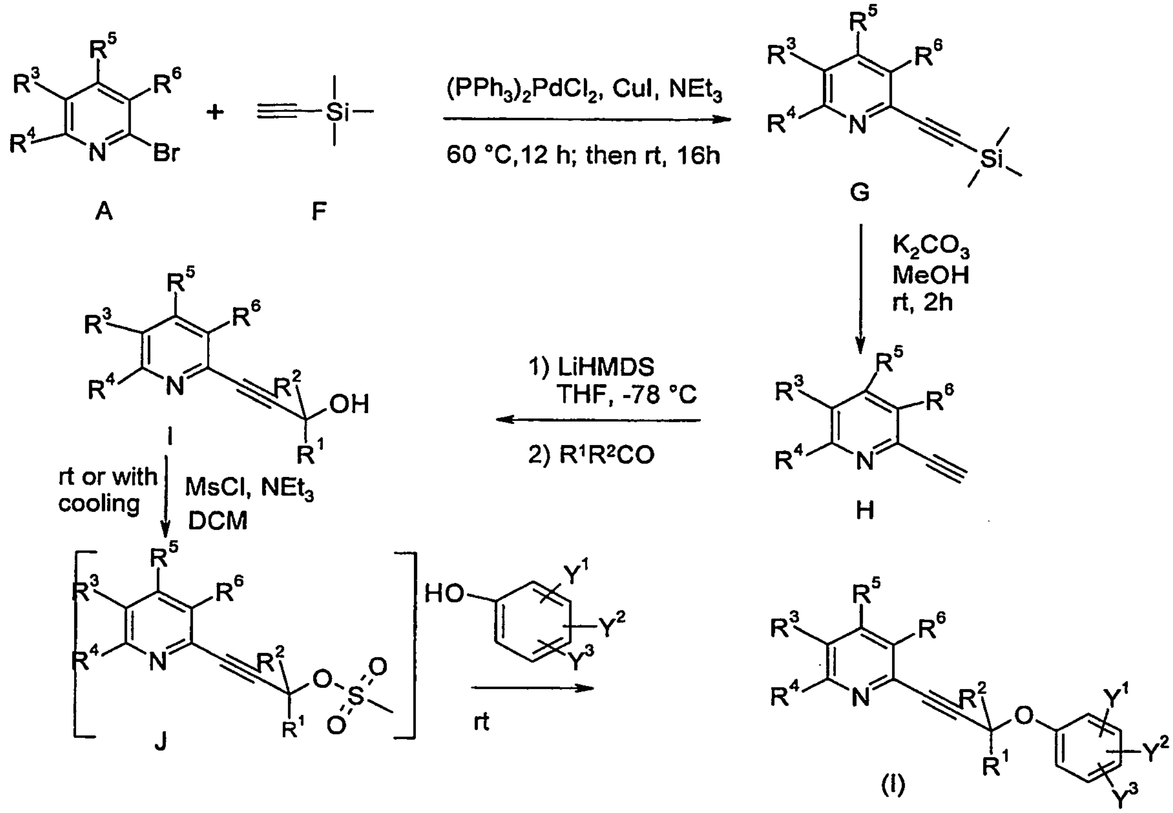
Methods of preparation

First, a Sonogashira coupling (*Tetrahedron Letters* 1975, 50, 4467, S. Thorand, N. Krause *J. Org. Chem.*, **1998**, *63*, 8551-8553, M. Erdélyi, A. Gogoll, *J. Org. Chem.*, **2001**, *66*, 4165-4169) of the aryl bromide A and the alcohol B in the presence of a base such as triethyl amine at room temperature to 60 °C gives the alcohol C which is then converted into the mesylate D with methanesulfonyl chloride in triethyl amine at about 0 to –20 °C. The mesylate of the primary alcohol is isolated and characterised, while that of the secondary alcohols are made in situ. Finally, the respective mesylate is reacted with the alcohol. This can either be done by adding the alcohol and a base such as triethyl amine to the mesylate in a solvent such as DCM or by pre-reacting the alcohol with a base such as sodium hydride in a solvent such as THF and subsequently adding the mesylate to this solution to generate product (I) (Scheme 1).



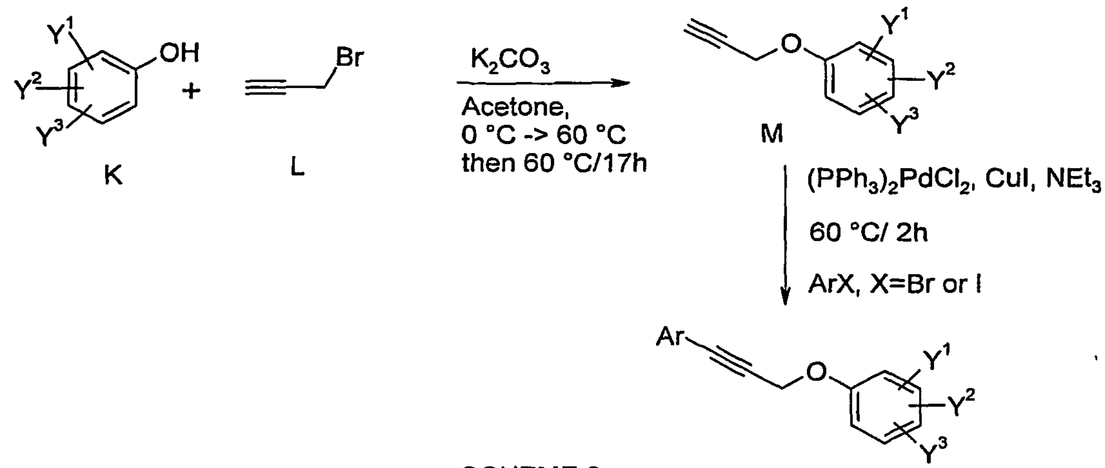
SCHEME 1

In those cases where the alcohol B is not commercially available with a desired R^1-group, the product (I) is formed by an alternative route (scheme 2): first the aryl bromide A is coupled with ethynyl(trimethyl)silane F via Sonogashira coupling at 60 °C in triethyl amine to give product G. Deprotection of G at room temperature with potassium carbonate in methanol/DCM gives terminal alkyne H, which is deprotonated with lithium bis(trimethylsilyl)amide in THF at – 78 °C. At – 78 °C an aldehyde or a ketone is added and the reaction mixture is allowed to reach room temperature and kept at that temperature for the appropriate time to form the alcohol I. Having isolated I, the mesylate J is formed in situ with methanesulfonyl chloride and triethyl amine, either at room temperature or with cooling. The reaction of the alcohol with this mesylate is either performed by adding the alcohol and a base such as triethyl amine to the mesylate in a solvent such as DCM or by pre-reacting the alcohol with a base such as sodium hydride in a solvent such as THF and subsequently adding the mesylate to this solution to form product (I).



SCHEME 2

Variations of compounds of formula I are investigated by first forming an aryl prop-2-yn-1-yl ether M by reaction of a phenol K with 3-bromoprop-1-yne [= propargyl bromide] L in the presence of a base such as potassium carbonate in e.g. acetone at temperatures from room temperature to 60 °C for the appropriate time (Scheme 3). The propargyl ether M is then reacted in a Sonogashira coupling with an aryl bromide in the presence of a base such as triethyl amine at temperatures from room temperature to 60 °C for the appropriate time.



SCHEME 3

In the schemes 1, 2 and 3 above, R^1, R^2, R^3, R^4, R^5, R^6, Y^1, Y^2 and Y^3 are defined as for the compounds of formula I above.

Experimental details

DCM is dried over 3Å molecular sieves. THF was distilled from Na/benzophenone just prior to use. All reactions are run under a nitrogen atmosphere. All glassware is dried in at 150 °C for at least two hours prior to its use. Phase separators from International Sorbent Technology (IST) are used. Purification by chromatography is done either on silica gel 60 (0.040-0.063 mm), or by reverse phase chromatography with a C8 column. All NMR spectra are measured in δ-chloroform.

2-bromo-6-methylpyridine is commercially available from Aldrich, $(PPh_3)_2PdCl_2$ from Avacado, Pd $(OAc)_2$ from Aldrich and CuI from Fluka. If not stated otherwise, the chemicals used are commercially available and are used as such without further purification.

Pharmaceutical formulations

For clinical use, the compounds of formula I are in accordance with the present invention suitably formulated into pharmaceutical formulations for oral administration. Also rectal, parenteral or any other route of administration may be contemplated to the skilled man in the art of formulations. Thus, the compounds of formula I are formulated with at least one pharmaceutically and pharmacologically acceptable carrier or adjuvant. The carrier may be in the form of a solid, semi-solid or liquid diluent.

In the preparation of oral pharmaceutical formulations in accordance with the invention, the compound of formula I to be formulated is mixed with solid, powdered ingredients such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable ingredient, as well as with disintegrating agents and lubricating agents such as magnesium stearate, calcium stearate, sodium stearyl fumarate and polyethylene glycol waxes. The mixture is then processed into granules or compressed into tablets.

Soft gelatine capsules may be prepared with capsules containing a mixture of the active compound or compounds of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatine capsules. Hard gelatine capsules may contain the active compound in combination with solid powdered ingredients such as lactose, saccharose, sorbitol, mannitol, potato starch, corn starch, amylopectin, cellulose derivatives or gelatine.

Dosage units for rectal administration may be prepared (i) in the form of suppositories which contain the active substance(s) mixed with a neutral fat base; (ii) in the form of a gelatine rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil, or other suitable vehicle for gelatine rectal capsules; (iii) in the form of a ready-made micro enema; or (iv) in the form of a dry micro enema formulation to be reconstituted in a suitable solvent just prior to administration.

Liquid preparations for oral administration may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions, containing the active compound and the remainder of the formulation consisting of sugar or sugar alcohols, and a mixture of ethanol, water, glycerol, propylene glycol and polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, saccharine and carboxymethyl cellulose or other thickening agent. Liquid preparations for oral administration may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

Solutions for parenteral administration may be prepared as a solution of a compound of the invention in a pharmaceutically acceptable solvent. These solutions may also contain stabilizing ingredients and/or buffering ingredients and are dispensed into unit doses in the form of ampoules or vials. Solutions for parenteral administration may also be prepared as a dry preparation to be reconstituted with a suitable solvent extemporaneously before use.

In one aspect of the present invention, the compounds of formula I may be administered once or twice daily, depending on the severity of the patient's condition.

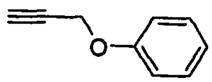
A typical daily dose of the compounds of formula I is from 0.1 – 10 mg per kg body weight of the subject to be treated, but this will depend on various factors such as the route of administration, the age and weight of the patient as well as of severity of the patient's condition.

Examples

Method K

Example 1

Preparation of 3-(prop-2-yn-1-yloxy)benzene (compound 21):

 (21)

Potassium carbonate (1.382 g, 0.1 mol) was added to a solution of phenol (0.941 g, 0.01 mmol, 1.0 eq.) in acetone (15 mL) at 0 °C. 3-bromoprop-1-yne (1.19 g, 0.89 mL, 0.01 mol, 1.0 eq.) was added. The solution was allowed to reach room temperature and then heated at 60 °C for 17h. After cooling, the solvent was evaporated.

Water (15 mL) was added and the mixture was extracted with EtOAc (3 x 15 mL). The combined organic phases were washed with water (1 x 15 mL), brine (1 x 15 mL), dried with magnesium sulphate and evaporated. This gave 1.026 g (78 %) of product.

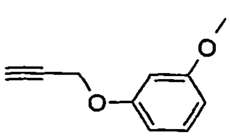
TLC: R_f (heptane/EtOAc 2:1) = 0.67.

^{1}H NMR (300 MHz): 7.32-7.24 (m, 2H), 7.02-6.92 (m, 3H), 4.64 (d, J = 2.4 Hz, 2H), 2.48 (t, J = 2.4 Hz, 1H).

^{13}C NMR (75 MHz): 157.6, 129.8, 121.7, 115.1, 78.9, 75.7, 56.0.

Example 2

Preparation of 1-methoxy-3-(prop-2-yn-1-yloxy)benzene (compound 22): according to method K above, with 3-methoxyphenol as starting material.

 (22)

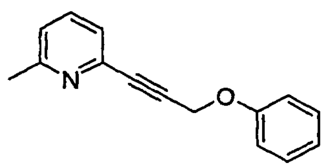
Yield: 99 %.

^{1}H NMR (500 MHz): 7.20-7.14 (m 2H), 6.57-6.51 (m, 3H), 4.63 (d, J = 2.3 Hz, 2H), 3.74 (s, 3H), 2.51 (t, J = 2.4 Hz, 1H).

^{13}C NMR (125 MHz): 160.8, 158.8, 129.9, 107.2, 106.9, 101.5, 78.6, 75.6, 55.8, 55.2.

Example 3

Preparation of 2-methyl-6-(3-phenoxyprop-1-yn-1-yl)pyridine (compound 23):



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To 2-bromo-6-methylpyridine (1.055 g, 6.13 mmol) was added 3-(prop-2-yn-1-yloxy)benzene (0.851 g, 6.44 mmol, 1.10 eq.), followed by $(PPh_3)_2PdCl_2$ (0.129 g, 0.18 mmol, 0.03 eq.), CuI (0.035 g, 0.18 mmol, 0.03 eq.) and triethylamine (3.50 mL). The mixture was heated under nitrogen at 60 °C for 2h. Phophate buffer (10 mL, 0.2 M, pH 7), was added and the water phase was extracted with DCM (3 x 10 mL). The combined organic phases were dried with magnesium sulphate, evaporated and then filtered through a Si-plug, 1 g, while rinsing with diethyl ether/pentane 1:1, ca. 25 mL. This gave 1.491g after evaporation.

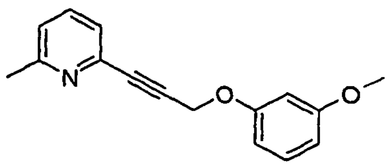
Flash chromatography on Si-gel with heptane/AcOEt, first 9:1, then 3:1, as eluent gave 0.908 g compound.

^{1}H NMR (500 MHz): 7.42 (t, J = 7.8 Hz, 1H), 7.27 (t, J = 7.8, 2H), 7.17 (d, J = 7.7 Hz, 1H), 7.01 (m, 3H), 6.95 (t, J = 7.7 Hz, 1H), 4,88 (s, 2H), 2.94 (s, 3H).

^{13}C NMR (125 MHz): 158.2, 157.1, 140.9, 135.8, 128.9, 123.7, 122.4, 120.8, 114.2, 85.8, 83.1, 55.7, 23.8.

Example 4

Preparation of 2-[3-(3-methoxyphenoxy)prop-1-yn-1-yl]-6-methylpyridine (compound 24):



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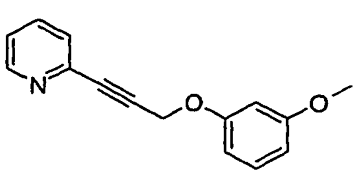
To NaH (0.038 g, 95 % purity, 1.50 mmol, 5.0 eq.) in THF (1 mL) was added 1-methoxy-3-(prop-2-yn-1-yloxy)benzene (0.037 g, 0.30 mmol,1.0 eq.) in THF (1 mL) at 0 °C under nitrogen. The mixture was stirred for 10 min. at room temperature. Then, 3-(6-methylpyridin-2-yl)prop-2-yn-1-yl methanesulfonate (0.068 g, 0.30 mmol) in THF (1 mL) was added at 0 °C. The mixture was stirred at room temperature over night (18 h). The mixture was poured onto water (10 mL) and the water phase was extracted with Et_2O (2 x 10 mL) and then DCM (2 x 10 mL). The combined organic phases were dried with sodium sulphate and evaporated. This gave 0.041 g crude product which was then purified by reverse phase chromatography. This gave 0.009 g (yield: 12 %) product.

^{1}H NMR (300 MHz): 7.52 (t, J = 7.8 Hz, 1H), 7.25 [m (under $CDCl_3$-signal), 1H), 7.20 (t, J = 8.2 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 6.66-6-53 (m, 3H), 4.91 (s, 2H), 3.79 (s, 3H), 2.55 (s, 3H).

Example 5

Preparation of 2-[3-(3-methoxyphenoxy)prop-1-yn-1-yl]pyridine (compound 25): prepared according to example 3 with 2-bromopyridine and 1-methoxy-3-(prop-2-yn-1-yloxy)benzene as starting materials.



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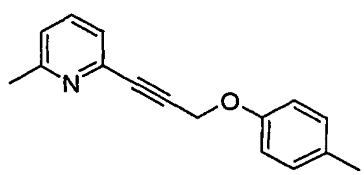
Example 6, Method A

Preparation of 2-Methyl-6- (3-p-tolyloxy-prop-1-ynyl)-pyridine

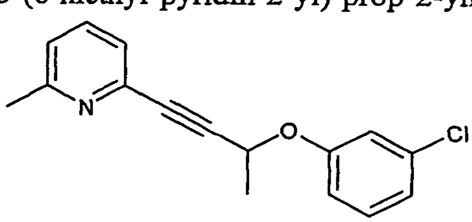


To a stirred mixture of 4-methyl-phenol (19mg, 0.18mmol) and K_2CO_3 (31mg, 0.225mmol) in acetone (0.3ml), methanesulfonic acid 3-(6-methyl-pyridin-2-yl)-prop-2-ynyl ester (34mg, 0.15mmol) dissolved in acetone (0.2ml) was added at room temperature. The mixture was stirred at 60°C for 18h. Since all solvent had evaporated but the reaction was not completed, dimethylformamide (0.5ml) was added and the reaction was stirred at 60°C for another 20h. The solid salt was filtered off and the filtrate was purified by HPLC: Waters FractionLynxsystem with UV, ELSD and MS. Column: Ace C8 5μ 100 mm x 21,2 mm id, mobile phase A: 95% acetonitrile, mobile phase B: 5% acetonitrile + 95% 0,1 M NH_4OAc Gradient: From 100% B to 100% A in 10 minutes, Flow: 25 ml/min UV: 254 nm. The title compound, 0.010g (yield 28%) was isolated. M+H: 238.1

Example 7

Preparation of 2-[3-(3-chlorophenoxy)but-1-yn-1-yl]-6-methylpyridine

The compound was prepared according to method A using methanesulfonic acid 1-methyl-3-(6-methyl-pyridin-2-yl)-prop-2-ynyl ester and 3-chlorophenol.

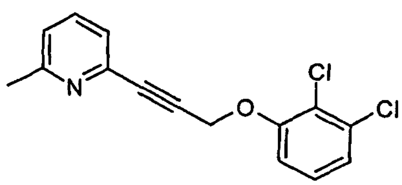


^{1}H NMR: 7.58 (s, 1H), 7.5 (t, 1H), 7.43 (m, 1H), 7.27 (m, 2H), 7.15 (d, 1H), 7.06 d, 1H), 4.14 (q, 1H), 2.52 (s, 3H), 1.62 (d, 3H)

^{13}C NMR: 159.0, 142.4, 136.5, 136.0, 134.6, 132.8, 131.2, 130.0, 128.2, 124.6, 122.9, 89.5, 83.8, 34.1, 24.7, 21.6.

Example 8

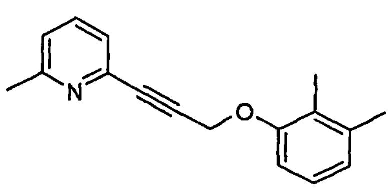
Preparation of 2-[3-(2,3-Dichloro-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,3-dichlorophenol (0.029g, 0.18mmol) 0.012g, (yield 27%) of the title compound was isolated. M+H: 292.0

Example 9

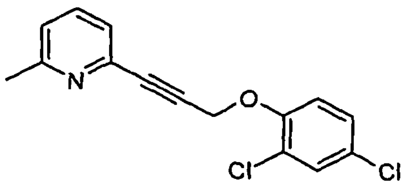
Preparation of 2-[3-(2,3-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,3-dimethylphenol (0.022g, 0.18mmol) 0.010g (yield 26%) of the title compound was isolated. M+H: 252.1

Example 10

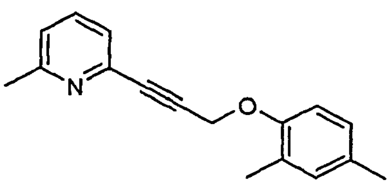
Preparation of 2-[3-(2,4-Dichloro-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,4-dichlorophenol (0.029g, 0.18mmol) 0.009g (yield 20%) of the title compound was isolated. M+H: 292.0

Example 11

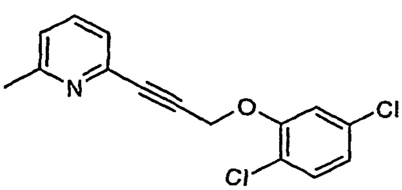
Preparation of 2-[3-(2,4-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,4-dimethylphenol (0.022g, 0.18mmol) 0.007g (yield 18%) of the title compound was isolated. M+H: 252.1

Example 12

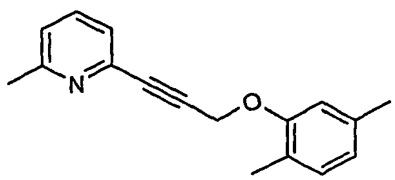
Preparation of 2-[3-(2,5-Dichloro-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,5-dichlorophenol (0.029g, 0.18mmol) 0.024g (yield 54%) of the title compound was isolated. M+H: 292.0

Example 13

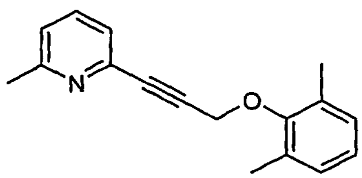
Preparation of 2-[3-(2,5-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,5-dimethylphenol (0.022g, 0.18mmol) 0.017g (yield 45%) of the title compound was isolated. M+H: 252.1

Example 14

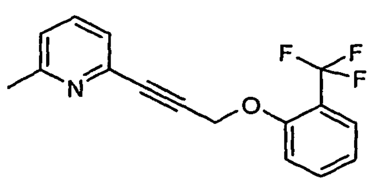
Preparation of 2-[3-(2,6-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2,6-dimethylphenol (0.022g, 0.18mmol) 0.019g (yield 50%) of the title compound was isolated. M+H: 252.1

Example 15

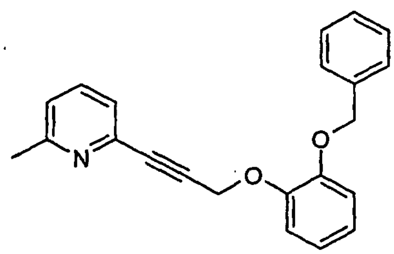
Preparation of 2-Methyl-6- [3-(2-trifluoromethyl-phenoxy)-prop-1-ynyl]-pyridine



Using method A starting from 2-trifluoromethylphenol (0.029g, 0.18mmol) 0.015g (yield 35%) of the title compound was isolated. M+H: 292.1

Example 16

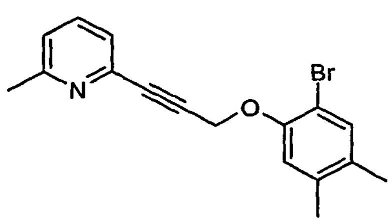
Preparation of 2-[3-(2-Benzyloxy-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from benzyl alcohol (0.036g, 0.18mmol) 0.016g (yield 32%) of the title compound was isolated. M+H: 330.2

Example 17

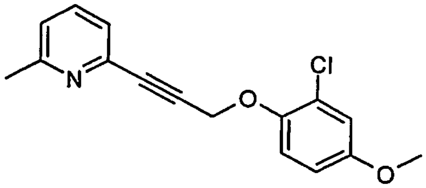
Preparation of 2-[3-(2-Bromo-4, 5-dimethyl-phenoxy) -prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2-bromo-4, 5-dimethylphenol (0.036g, 0.18mmol) 0.017g (yield 33%) of the title compound was isolated. M+H: 330.1

Example 18

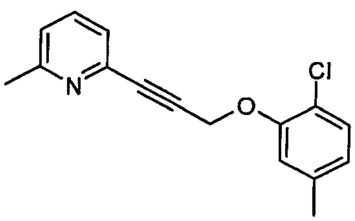
Preparation of 2-[3-(2-Chloro-4-methoxy-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2-chloro-4-methoxyphenol (0.029g, 0.18mmol) 0.022g (yield 50%) of the title compound was isolated. M+H: 288.1

Example 19

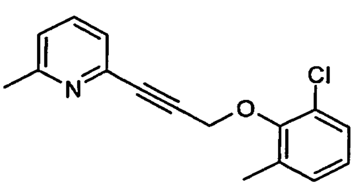
Preparation of 2-[3-(2-Chloro-5-methyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2-chloro-5-methylphenol (0.026g, 0.18mmol) 0.021g (yield 53%) of the title compound was isolated. M+H: 272.1

Example 20

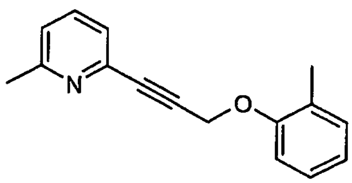
Preparation of 2-[3-(2-Chloro-6-methyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 2-chloro-6-methylphenol (0.026g, 0.18mmol) 0.017g (yield 42%) of the title compound was isolated. M+H: 272.1

Example 21

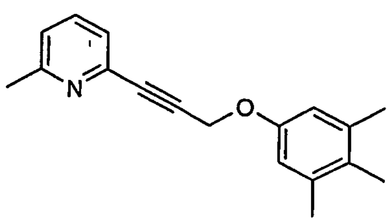
Preparation of 2-Methyl-6- (3-o-tolyloxy-prop-1-ynyl)-pyridine



Using method A starting from 2-methylphenol (0.019g, 0.18mmol) 0.017g (yield 47%) of the title compound was isolated. M+H: 238.1

Example 22

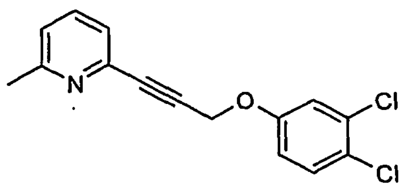
Preparation of 2-Methyl-6- [3-(3,4,5-trimethyl-phenoxy)-prop-1-ynyl]-pyridine



Using method A starting from 3,4,5-trimethyl phenol (0.025g, 0.18mmol) 0.012g (yield 30%) of the title compound was isolated. M+H: 266.1

Example 23

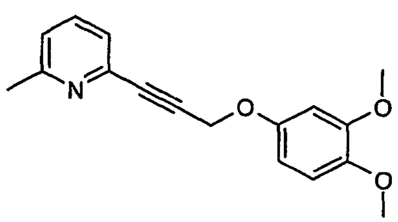
Preparation of 2-[3-(3,4-Dichloro-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,4-dichloro phenol (0.029g, 0.18mmol) 0.021g (yield 49%) of the title compound was isolated. M+H: 292.0

Example 24

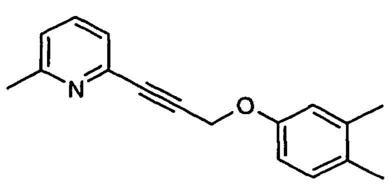
Preparation of 2-[3-(3,4-Dimethoxy-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,4-dimethoxy phenol (0.028g, 0.18mmol) 0.017g (yield 39%) of the title compound was isolated. M+H: 284.1

Example 25

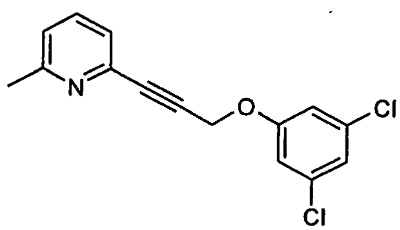
Preparation of 2-[3-(3,4-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,4-dimethyl phenol (0.022g, 0.18mmol) 0.012g (yield 32%) of the title compound was isolated. M+H: 252.1

Example 26

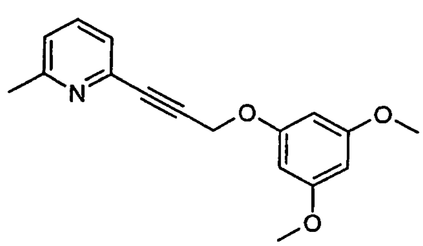
Preparation of 2-[3-(3,5-Dichloro-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,5-dichlorophenol (0.029g, 0.18mmol) 0.015g (yield 34%) of the title compound was isolated. M+H: 292.0

Example 27

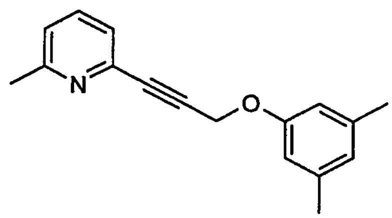
Preparation of 2-[3-(3,5-Dimethoxy-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,5-dimethoxyphenol (0.028g, 0.18mmol) 0.014g (yield 33%) of the title compound was isolated. M+H: 284.1

Example 28

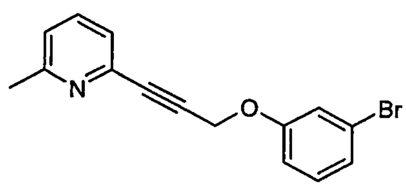
Preparation of 2-[3-(3,5-Dimethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3,5-dimethylphenol (0.022g, 0.18mmol) 0.018g (yield 47%) of the title compound was isolated. M+H: 252.1

Example 29

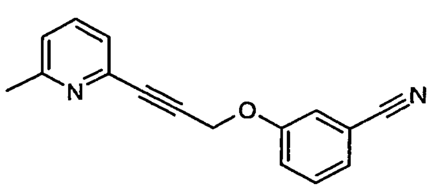
Preparation of 2-[3-(3-Bromo-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3-bromophenol (0.031g, 0.18mmol) 0.020g (yield 45%) of the title compound was isolated. M+H: 302.0

Example 30

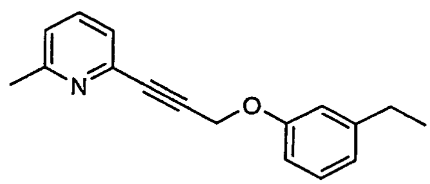
Preparation of 3- [3- (6-Methyl-pyridin-2-yl)-prop-2-ynyloxy]-benzonitrile



Using method A starting from 3-hydroxybenzonitrile (0.021g, 0.18mmol) 0.010g (yield 27%) of the title compound was isolated. M+H: 249.1

Example 31

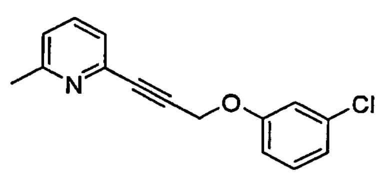
Preparation of 2-[3-(3-Ethyl-phenoxy)-prop-1-ynyl]-6-methyl-pyridine



Using method A starting from 3-ethylphenol (0.022g, 0.18mmol) 0.030g (yield 79%) of the title compound was isolated. M+H: 252.1

Example 32

Preparation of 2-methyl-6-[3-(3-methylphenoxy)prop-1-yn-1-yl]pyridine



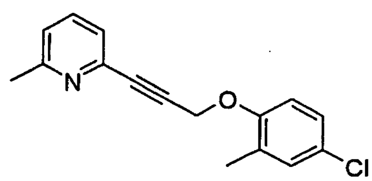
To 3-chlorophenol (0.031 g, 0.24 mmol, 1.2 eq.) in a glass vial was added anhydrous potassium carbonate (0.041 g, 0.30 mmol, 1.5 eq.), followed by 0.5 mL of a 0.4 M solution of 3-(6-methylpyridin-2-yl)prop-2-yn-1-yl methanesulfonate (0.045 g, 0.2 mmol) in acetone. The vial was sealed and heated at 60 °C for 5 h. The material was filtered through Celite and then vacuum centrifuged. Purification was done by reverse phase column chromatography. Yield: 0.023 g (40 %).

^1H NMR (500 MHz): 7.53 (t, J = 7.8 Hz, 1H), 7.27-7.20 (m, ca. 2H, overlap with $CDCl_3$ signal), 7.11 (br d, J = 8.0 Hz, 1H), 7.04-7.02 (m, 1H), 6.98 (br d, J = 7.8 Hz, 1H), 6.92 (br d, J = 8.4 Hz, 1H), 4.92 (s, 2H), 2.55 (s, 3H).

MS $^m/_z$: 258 (M+1)

Example 33

Preparation of 2-[3-(4-chloro-2-methylphenoxy)prop-1-yn-1-yl]-6-methylpyridine:

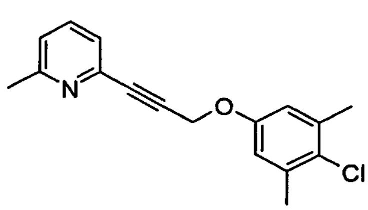


Prepared in analogy to example 32, but with 4-chloro-2-methylphenol as starting material. Yield: 0.008 g (20 %).

MS $^m/_z$: 272 (M+1)

Example 34

Preparation of 2-[3-(4-chloro-3,5-dimethylphenoxy)prop-1-yn-1-yl]-6-methylpyridine:

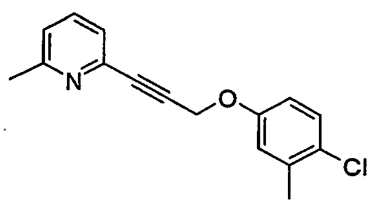


Prepared in analogy to example 32, but in 0.15 mmol scale and with 4-chloro-3,5-dimethylphenol as starting material. Yield: 0.007 g (16 %).

MS $^{m}/_{z}$: 286 (M+1)

Example 35

Preparation of 2-[3-(4-chloro-3-methylphenoxy)prop-1-yn-1-yl]-6-methylpyridine:

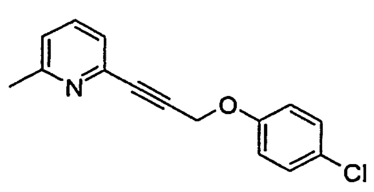


Prepared in analogy to example 32, but in 0.15 mmol scale and with 4-chloro-3-methylphenol as starting material. Yield: 0.010 g (25 %).

MS $^{m}/_{z}$: 272 (M+1)

Example 36

Preparation of 2-[3-(4-chlorophenoxy)prop-1-yn-1-yl]-6-methylpyridine:

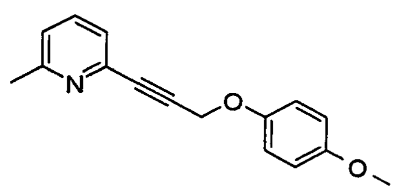


Prepared in analogy to example 32, but in 0.15 mmol scale and with 4-chlorophenol as starting material. Yield: 0.008 g (21 %).

MS $^{m}/_{z}$: 258 (M+1)

Example 37

Preparation of 2-[3-(4-methoxyphenoxy)prop-1-yn-1-yl]-6-methylpyridine:

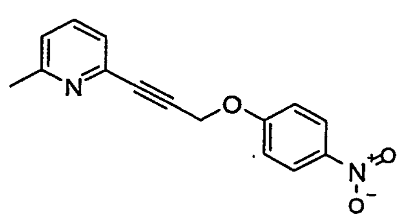


Prepared in analogy to example 32, but in 0.15 mmol scale and with 4-methoxyphenol as starting material. Yield: 0.004 g (11 %).

MS $^{m}/_{z}$: 254 (M+1)

Example 38

Preparation of 2-methyl-6-[3-(4-nitrophenoxy)prop-1-yn-1-yl]pyridine:

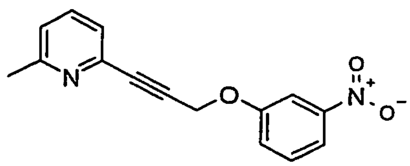


Prepared in analogy to example 32, but in 0.15 mmol scale and with 4-nitrophenol as starting material. Yield: 0.014 g (35 %).

MS $^{m}/_{z}$: 269 (M+1)

Example 39

Preparation of 2-methyl-6-[3-(3-nitrophenoxy)prop-1-yn-1-yl]pyridine:

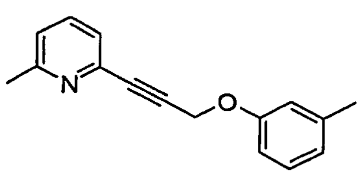


Prepared in analogy to example 32, but in 0.15 mmol scale and with 3-nitrophenol as starting material. Yield: 0.012 g (30 %).

MS $^{m}/_{z}$: 269 (M+1)

Example 40

Preparation of 2-methyl-6-[3-(3-methylphenoxy)prop-1-yn-1-yl]pyridine:



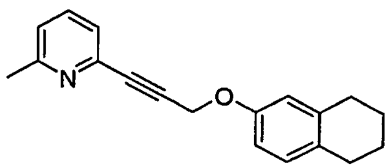
Prepared in analogy to example 32, but with 3-methylphenol as starting material. Yield: 0.012 (25 %).

^{1}H NMR (500 MHz): 7.52 (t, J = 7.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.21-7.16 (m, 1H), 7.10 (d, J = 7.8 Hz, 1H), 6.85-6.79 (m, 3H), 4.91 (s, 2H), 2.55 (s, 3H), 2.34 (s, 3H).

MS $^{m}/_{z}$: 238 (M+1)

Example 41

Preparation of 2-methyl-6-[3-(5,6,7,8-tetrahydronaphthalen-2-yloxy)prop-1-yn-1-yl]pyridine:

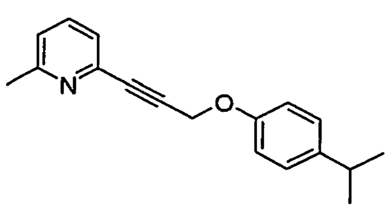


Prepared in analogy to example 32, but with 5,6,7,8-tetrahydronaphthalen-2-ol as starting material. Yield: 0.021 g (38 %).

^{1}H NMR (300 MHz): 7.52 (t, J = 7.8 Hz, 1H), 7.25 (d, J = ca. 7.8 Hz, 1H, overlap with $CDCl_3$ signal), 7.10 (d, J = 7.8 Hz, 1H), 6.99 (d, J = 8.3 Hz, 1H), 6.85-6.70 (m, 2H), 4.88 (s, 2H), 2.78-2.67 (m, 4H), 2.55 (s, 3H), 1.80-1.74 (m, 4H).
MS $^m/_z$: 278 (M+1)

Example 42

Preparation of 2-[3-(4-isopropylphenoxy)prop-1-yn-1-yl]-6-methylpyridine:

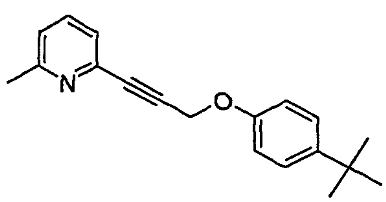


Prepared in analogy to example 32, but with 4-isopropylphenol as starting material. Yield: 0.046 (85 %).

^{1}H NMR (500 MHz): 7.52 (t, J = 7.8 Hz, 1H), 7.25 (d, J = ca. 7.7 Hz, 1H, overlap with $CDCl_3$ signal), 7.16 (d, J = 8.4 Hz, 2H), 7.10 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 8.4 Hz, 2H), 4.90 (s, 2H), 2.90-2.83 (m, J = 7.0 Hz, 1H), 2.55 (s, 3H), 1.23 (d, J = 7.0 Hz, 6H).
MS $^m/_z$: 266 (M+1)

Example 43

Preparation of 2-[3-(4-*tert*-butylphenoxy)prop-1-yn-1-yl]-6-methylpyridine:



Prepared in analogy to example 32, but with 4-*tert*-butylphenol as starting material. Yield: 0.017 g (30 %).

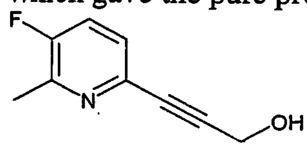
^{1}H NMR (500 MHz): 7.52 (t, J = 7.8 Hz, 1H), 7.32 (br d, J = 8.8 Hz, 2H), 7.25 (m, partly overlap with $CDCl_3$ signal, 1H(?)), 7.10 (d, J = 7.8 Hz, 1H), 6.96 (br d, J = 8.8 Hz, 2H), 4.90 (s, 2H), 2.55 (s, 3H), 1.30 (s, 9H).
MS $^m/_z$: 280 (M+1)

Example 44

Preparation of 3-(5-fluoro-6-methylpyridin-2-yl)prop-2-yn-1-ol:

6-Bromo-3-fluoro-2-methylpyridine (0.500 g, 2.63 mmol), propargyl alcohol (0.590 g, 10.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (46 mg, 0.065 mmol) were dissolved in triethylamine (1.0 mL) and finally copper(I) iodide was added. The mixture was slowly heated to 50 °C and left under stirring overnight. Hereafter K_2CO_3 (1.0 M, 25 mL) was added and the solution was extracted with DCM. The organic phases were

pooled, dried (Na_2SO_4), filtrated and evaporated. The resulting crude product was subjected to flash chromatography on silica gel with gradient (heptane/EtOAc 1:0 to 1:4), which gave the pure product 0.332 g (76 %).

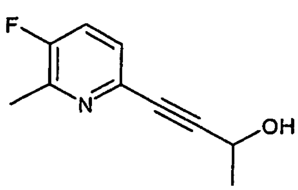


^{1}H-NMR (400 MHz): 7.22 (d, 1H), 7.20 (s, 1H), 4.57 (m, 1H), 4.47 (m, 2H), 2.43 (d, 3H). ^{13}C-NMR (400 MHz): 158.7, 156.1, 147.6 (d), 137.9 (d), 126.4 (d), 122.9 (d), 88.2, 83.4, 50.9, 17.8.

Example 45

Preparation of 4-(5-fluoro-6-methylpyridin-2-yl)but-3-yn-2-ol:

6-Bromo-3-fluoro-2-methylpyridine (0.500 g, 2.63 mmol), 3-butyn-2-ol (0.738 g, 10.5 mmol) and bis(triphenylphosphine)palladium(II) chloride (46 mg, 0.065 mmol) were dissolved in triethylamine (1.0 mL) and finally copper(I) iodide was added. The mixture was slowly heated to 50 °C and left under stirring overnight and then heated to 70 °C for additionally 2 h. Hereafter K_2CO_3 (1.0 M, 25 mL) was added and the solution was extracted with DCM. The organic phases were pooled, dried (Na_2SO_4), filtrated and evaporated. The resulting crude product was subjected to flash chromatography on silica gel with gradient (heptane/EtOAc 1:0 to 1:4), which gave the pure product 0.447 g (95 %).

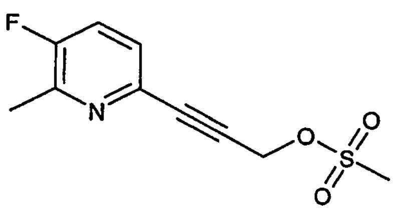


^{1}H-NMR (400 MHz): 7.18 (s, 1H), 7.16 (s, 1H), 4.72 (m, 1H), 4.45 (m, 1H), 2.41 (d, 3H), 1.47 (d, 3H). ^{13}C-NMR (400 MHz): 158.6, 156.0, 147.5 (d), 138.0 (d), 126.3 (d), 122.9 (d), 91.5, 82.1, 58.2, 24.1, 17.8.

Example 46

Preparation of 3-(5-fluoro-6-methylpyridin-2-yl)prop-2-yn-1-yl methanesulfonate:

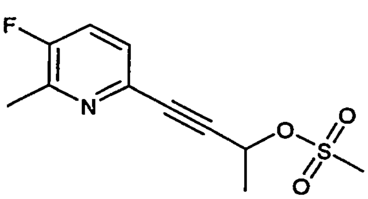
3-(5-fluoro-6-methylpyridin-2-yl)prop-2-yn-1-ol (40.0 mg, 0.24 mmol) and diisopropylethylamine (125 mg, 0.97 mmol) were dissolved in DCM (1.0 mL) and mesyl chloride was added dropwise. The mixture was stirred for 2 h at room temperature. The reaction was quenched with water (25 mL) and extracted with DCM. The organic phases were pooled, dried (Na_2SO_4), filtrated and evaporated. The crude material was sufficiently pure (LC-MS) to be used for further synthesis.



Example 47

Preparation of 3-(5-fluoro-6-methylpyridin-2-yl)-1-methylprop-2-yn-1-yl methanesulfonate:

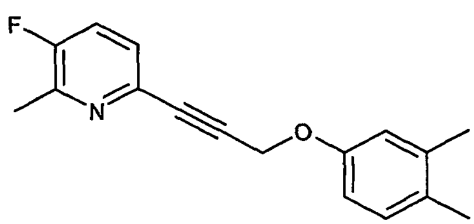
4-(5-fluoro-6-methylpyridin-2-yl)but-3-yn-2-ol (43.4 mg, 0.24 mmol) and diisopropylethylamine (125 mg, 0.97 mmol) were dissolved in DCM (1.0 mL) and mesyl chloride (55.4 mg, 0.48 mmol) was added dropwise. The mixture was stirred for 2 h at room temperature. The reaction was quenched with water (25 mL) and extracted with DCM. The organic phases were pooled, dried (Na_2SO_4), filtrated and evaporated. The crude material was sufficiently pure (LC-MS) to be used for further synthesis.



Example 48

Preparation of 6-[3-(3,4-dimethylphenoxy)prop-1-yn-1-yl]-3-fluoro-2-methylpyridine:

3-(5-fluoro-6-methylpyridin-2-yl)prop-2-yn-1-yl methanesulfonate (10.0 mg, 0.041 mmol) and 3,4-dimethylphenol (7.5 mg, 0.061 mmol) were dissolved in acetone/DMF (2:1, 3 mL) and K_2CO_3 (11.4 mg, 0.082 mmol) was added. The mixture was heated to 60 °C overnight. Hereafter K_2CO_3 (1.0 M, 25 mL) was added and the solution was extracted with DCM. The organic phases were pooled, dried (Na_2SO_4), filtrated and evaporated. The resulting crude product was purified by preparative HPLC (C_8 kromasil), which afforded the pure product 2.0 mg (9.0 %).

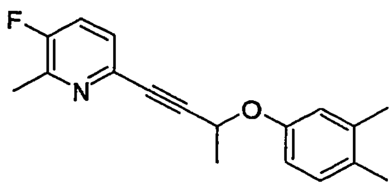


^{1}H-NMR (400 MHz): 7.26 (m, 2H), 7.04 (d, 1H), 6.76 (dd, 2H), 4.87 (s, 2H), 2.51 (d, 3H), 2.24 (s, 3H), 2.19 (s, 3H).

Example 49

Preparation of 6-[3-(3,4-dimethylphenoxy)but-1-yn-1-yl]-3-fluoro-2-methylpyridine:

3-(5-fluoro-6-methylpyridin-2-yl)-1-methylprop-2-yn-1-yl methanesulfonate (62.3 mg, 0.242 mmol) and 3,4-dimethylphenol (44.4 mg, 0.363 mmol) were dissolved in acetone/DMF (2:1, 3 mL) and K_2CO_3 (66.9 mg, 0.484 mmol) was added. The mixture was heated to 60 °C overnight. Hereafter K_2CO_3 (1.0 M, 25 mL) was added and the solution was extracted with DCM. The organic phases were pooled, dried (Na_2SO_4), filtrated and evaporated. The resulting crude product was purified by preparative HPLC (C_8 kromasil), which afforded the pure product 22.0 mg (32 %).



^{1}H-NMR (400 MHz): 7.22 (m, 2H), 7.03 (d, 1H), 6.82 (m, 2H), 5.04 (q, 1H), 2.50 (d, 3H), 2.23 (s, 3H), 2.19 (s, 3H), 1.71 (d, 3H)

Example 50

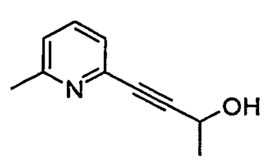
Preparation of 4-(6-methylpyridin-2-yl)but-3-yn-2-ol:

2-bromo-6-methylpyridine (0.258 g, 1.5 mmol) was mixed with but-3-yn-2-ol (0.116 g, 1.65 mmol, 1.1 eq.) and $(PPh_3)_2PdCl_2$ (0.032 g, 0.045 mmol, 0.03 eq.). At 0 °C triethylamine (0.61 g, 0.84 mL, 6.0 mmol, 4.0 eq.) was added. The mixture was stirred at 0 °C for 10 min and CuI (0.006 g, 0.03 mmol, 0.02 eq.) was added. The mixture was allowed to reach room temperature and was finally heated at 60 °C for 4h.

Phosphate buffer (10 mL, 0.2 M, pH 7) was added and the water phase was extracted with DCM (3x10 mL) by using a phase separator. The combined organic phases were dried with sodium sulphate and evaporated. This gave 0.286 g crude product.

After flash chromatography on Si with pentane/EtOAc fractions (first 1:1, then 3:2 and finally 1:2) as eluent 0.163 g (Yield: 67 %) pure product was isolated as a yellow oil.

TLC: R_f (pentane/EtOAc 1:1) = 0.20.



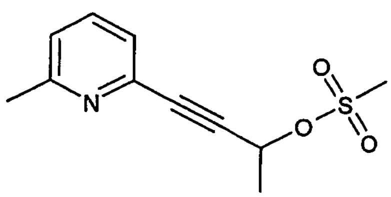
^{1}H NMR (300 MHz): 7.40 (t, J = 7.8 Hz, 1H), 7.10 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 7.8 Hz, 1H), 4.90 (b, 1H), 4.76 (q, J = 6.8 Hz, 1H), 2.43 (s, 3H), 1.49 (d, J = 6.7 Hz, 3H).

^{13}C NMR (75 MHz): 158.2, 141.7, 136.2, 123.9, 122.4, 91.7, 82.3, 57.6, 23.9, 23.8.

Example 51

Preparation of Methanesulfonic acid 1-methyl-3-(6-methyl-pyridin-2-yl)-prop-2-ynyl ester

The compound was prepared according to the method in example 46 using 4-(6-methylpyridin-2-yl)but-3-yn-2-ol as starting material.

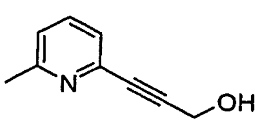


MS $^{m}/_{z}$: 240 (M+1)

Example 52

Preparation of 3-(6-methylpyridin-2-yl)prop-2-yn-1-ol:

To 2-bromo-6-methylpyridine (1.72 g, 0.01 mol) was added $(PPh_3)_2PdCl_2$ (0.116 g, 0.2 mmol, 0.02 eq.) and CuI (0.063 g, 0.3 mmol, 0.03 eq.) at 0 °C under nitrogen, followed by prop-2-yn-1-ol (2.24 g, 2.33 mL, 0.4 mol, 4.0 eq.) and triethylamine (1.50 mL). The reaction mixture was allowed to reach room temperature and then heated at 60 °C for 3.5 h. Then the reaction mixture was added to water (10 mL) and the pH was adjusted to 6-7 with 2 M HCl. The water phase was extracted with DCM (3 x 10 mL) and the combined organic phases were dried with sodium sulphate and evaporated. This gave 1.719 g crude product. 1.098 g hereof was subjected to flash chromatography on silica gel with pentane/EtOAc, first 1:1, then 1:2, finally 1:3, as eluent. This gave 0.578 g product.

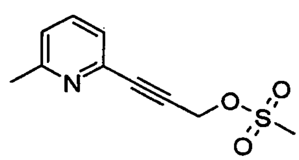


^{13}C NMR (75 MHz): 157.7, 141.1, 136.2, 123.7, 122.3, 88.4, 83.0, 49.9, 23.5.

Example 53

Preparation of 3-(6-methylpyridin-2-yl)prop-2-yn-1-yl methanesulfonate:

3-(6-methylpyridin-2-yl)prop-2-yn-1-ol (0.300 g, 2.04 mmol) was dissolved in DCM (10 mL) under nitrogen over 5-10 min. The solution was cooled to -20 °C (cooling bath: acetone + pieces of dry ice). Triethylamine (0.268 g, 0.37 mL, 0.27 mmol, 1.30 eq.) was added. Methanesulfonyl chloride (0.280 g, 0.19 mL, 0.24 mmol, 1.2 eq.) in DCM (1.5 mL) was added over 3 min. The reaction mixture was stirred at -18 to -22 °C for 1h after which time LC/MS only showed product. Water (10 mL) was added. The organic phase was separated and the water phase was extracted with DCM (3 x 10 mL). The organic phases were pooled, dried with magnesium sulphate and evaporated. This gave 0.450 g (yield: 98 %) as a yellow oil.



^{1}H NMR (300 MHz): 7.61 (t, J = 7.7 Hz, 1H), 7.31 (d, J =7.7 Hz,1H), 7.19 (d, J = 7.7 Hz, 1H), 5.10 (s, 2H), 3.18 (s, 3H), 2.58 (s, 3H).

^{13}C NMR (75 MHz): 158.9, 140.2, 136.8, 124.5, 123.8, 87.8, 80.7, 57.7, 38.9, 24.2.

Biological evaluation

Functional assessment of mGluR5 antagonism in cell lines expressing mGluR5d

The properties of the compounds of the invention can be analyzed using standard assays for pharmacological activity. Examples of glutamate receptor assays are well known in the art as described in for example Aramori *et al., Neuron* 8:757 (1992), Tanabe *et* al., *Neuron* 8:169 (1992), Miller *et al., J. Neuroscience* 15: 6103 (1995), Balazs, *et al., J. Neurochemistry* 69:151 (1997). The methodology described in these publications is incorporated herein by reference. Conveniently, the compounds of the invention can be studied by means of an assay (FLIPR) that measures the mobilization of intracellular

calcium, $[Ca^{2+}]_i$ in cells expressing mGluR5 or another assay (IP3) that measures inositol phosphate turnover.

FLIPR Assay

Cells expressing human mGluR5d as described in WO97/05252 are seeded at a density of 100,000 cells per well on collagen coated clear bottom 96-well plates with black sides and experiments are done 24 h following seeding. All assays are done in a buffer containing 127 mM NaCl, 5 mM KCl, 2 mM $MgCl_2$, 0.7 mM NaH_2PO_4, 2 mM $CaCl_2$, 0.422 mg/ml $NaHCO_3$, 2.4 mg/ml HEPES, 1.8 mg/ml glucose and 1 mg/ml BSA Fraction IV (pH 7.4). Cell cultures in the 96-well plates are loaded for 60 minutes in the above mentioned buffer containing 4 μM of the acetoxymethyl ester form of the fluorescent calcium indicator fluo-3 (Molecular Probes, Eugene, Oregon) in 0.01% pluronic acid (a proprietary, non-ionic surfactant polyol – CAS Number 9003-11-6). Following the loading period the fluo-3 buffer is removed and replaced with fresh assay buffer. FLIPR experiments are done using a laser setting of 0.800 W and a 0.4 second CCD camera shutter speed with excitation and emission wavelengths of 488 nm and 562 nm, respectively. Each experiment is initiated with 160 μl of buffer present in each well of the cell plate. A 40 μl addition from the antagonist plate was followed by a 50 μL addition from the agonist plate. A 90 second interval separates the antagonist and agonist additions. The fluorescence signal is sampled 50 times at 1 second intervals followed by 3 samples at 5 second intervals immediately after each of the two additions. Responses are measured as the difference between the peak height of the response to agonist, less the background fluorescence within the sample period. IC_{50} determinations are made using a linear least squares fitting program.

IP3 Assay

An additional functional assay for mGluR5d is described in WO97/05252 and is based on phosphatidylinositol turnover. Receptor activation stimulates phospholipase C activity and leads to increased formation of inositol 1,4,5,triphosphate (IP_3).

GHEK stably expressing the human mGluR5d are seeded onto 24 well poly-L-lysine coated plates at 40 x 10^4 cells /well in media containing 1 μCi/well [3H] myo-inositol.

Cells were incubated overnight (16 h), then washed three times and incubated for 1 h at 37°C in HEPES buffered saline (146 mM NaCl, 4.2 mM KCl, 0.5 mM $MgCl_2$, 0.1% glucose, 20 mM HEPES, pH 7.4) supplemented with 1 unit/ml glutamate pyruvate transaminase and 2 mM pyruvate. Cells are washed once in HEPES buffered saline and pre-incubated for 10 min in HEPES buffered saline containing 10 mM LiCl. Compounds are incubated in duplicate at 37°C for 15 min, then either glutamate (80 μM) or DHPG (30 μM) is added and incubated for an additional 30 min. The reaction is terminated by the addition of 0.5 ml perchloric acid (5%) on ice, with incubation at 4°C for at least 30 min. Samples are collected in 15 ml polyproplylene tubes and inositol phosphates are separated using ion-exchange resin (Dowex AG1-X8 formate form, 200-400 mesh, BIORAD) columns. Inositol phosphate separation was done by first eluting glycero phosphatidyl inositol with 8 ml 30 mM ammonium formate. Next, total inositol phosphates is eluted with 8 ml 700 mM ammonium formate / 100 mM formic acid and collected in scintillation vials. This eluate is then mixed with 8 ml of scintillant and [3H] inositol incorporation is determined by scintillation counting. The dpm counts from the duplicate samples are plotted and IC_{50} determinations are generated using a linear least squares fitting program.

Abbreviations

BSA	Bovine Serum Albumin
CCD	Charge Coupled Device
CRC	Concentration Response Curve
DHPG	3,5-dihydroxyphenylglycine
DPM	Disintegrations per Minute
EDTA	Ethylene Diamine Tetraacetic Acid
FLIPR	Fluorometric Imaging Plate reader
GHEK	GLAST-containing Human Embrionic Kidney
GLAST	glutamate/aspartate transporter
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (buffer)
IP_3	inositol triphosphate

Generally, the compounds are active in the assay above with IC_{50} values less than 10 000 nM. In one aspect of the invention, the IC_{50} value is less than 1 μM. In a further aspect of the invention, the IC_{50} value is less than 100 nM.

Examples of IC_{50} values for individual compounds is given below:

Compound	**FLIPR IC_{50}**
3- [3- (6-Methyl-pyridin-2-yl)-prop-2-ynyloxy]-benzonitrile	545
2-methyl-6-[3-(4-nitrophenoxy)prop-1-yn-1-yl]pyridine	268
6-[3-(3,4-dimethylphenoxy)prop-1-yn-1-yl]-3-fluoro-2-methylpyridine	177

Screening for compounds active against TLESR

Adult Labrador retrievers of both genders, trained to stand in a Pavlov sling, are used. Mucosa-to-skin esophagostomies are formed and the dogs are allowed to recover completely before any experiments are done.

Motility measurement

In brief, after fasting for approximately 17 h with free supply of water, a multilumen sleeve/sidehole assembly (Dentsleeve, Adelaide, South Australia) is introduced through the esophagostomy to measure gastric, lower esophageal sphincter (LES) and esophageal pressures. The assembly is perfused with water using a low-compliance manometric perfusion pump (Dentsleeve, Adelaide, South Australia). An air-perfused tube is passed in

the oral direction to measure swallows, and an antimony electrode monitored pH, 3 cm above the LES. All signals are amplified and acquired on a personal computer at 10 Hz.

When a baseline measurement free from fasting gastric/LES phase III motor activity has been obtained, placebo (0.9% NaCl) or test compound is administered intravenously (i.v., 0.5 ml/kg) in a foreleg vein. Ten min after i.v. administration, a nutrient meal (10% peptone, 5% D-glucose, 5% Intralipid, pH 3.0) is infused into the stomach through the central lumen of the assembly at 100 ml/min to a final volume of 30 ml/kg. The infusion of the nutrient meal is followed by air infusion at a rate of 500 ml/min until an intragastric pressure of 10±1 mmHg is obtained. The pressure is then maintained at this level throughout the experiment using the infusion pump for further air infusion or for venting air from the stomach. The experimental time from start of nutrient infusion to end of air insufflation is 45 min. The procedure has been validated as a reliable means of triggering TLESRs.

TLESRs is defined as a decrease in lower esophageal sphincter pressure (with reference to intragastric pressure) at a rate of >1 mmHg/s. The relaxation should not be preceded by a pharyngeal signal ≤2s before its onset in which case the relaxation is classified as swallow-induced. The pressure difference between the LES and the stomach should be less than 2 mmHg, and the duration of the complete relaxation longer than 1 s.